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The overall goal of the proposed studies is to further elucidate the mechanisms by which the brainstem noradrenergic (NA) nucleus, locus coeruleus (LC), is capable of altering forebrain electrophysiological activity.

The proposed studies have the following Specific Aims: 1) To examine the relationship between the intensity of LC neuronal activity, forebrain EEG activation, and rates of NA release in neocortex and hippocampus using microdialysis; 2) To test the hypothesis that LC-induced activation of forebrain EEG is mediated by LC/NA actions on septal and basal forebrain neurons; 3) To examine, in μnanesthetized monkey, the effects of activating or inactivating the LC/NA system on forebrain EEG and on dialysis measures of NA and acetylcholine release in neocortex and hippocampus. The effects on these dialysis measures of systemic adrenergic drugs that alter cognitive performance will also be determined; 4) To examine, in monkey, the effects of activating or inactivating the LC/NA system on cortical and hippocampal EEG measures and on complex, bimanual motor behavior.

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TITLE: Extrathalamic Modulation of Cortical Responsiveness

PRINCIPAL INVESTIGATOR: Stephen L. Foote

DATE: 31 July, 1994

Organization of Technical Report. This report, which describes progress during the current 1-year funding period (Year 07), is divided into four sections that correspond to the Specific Aims for Years 07-09 of our project, with an additional section for studies relevant to the overall aims of the project but not previously specifically proposed. Full-length reports that were either accepted for publication or actually published during Year 07 are listed at the end of the Technical Report. The numbers in square brackets within the text of the report refer to the corresponding publications from this list.

AIM 1: TO EXAMINE THE RELATIONSHIPS AMONG LOCUS COERULEUS (LC) NEURONAL ACTIVITY, FOREBRAIN ELECTROENCEPHALOGRAPHIC (EEG) MEASURES, AND RATES OF NORADRENALINE (NA) RELEASE IN NEOCORTEX AND HIPPOCAMPUS BY PERFORMING MICRODIALYSIS AND OBTAINING EEG MEASURES IN THESE FOREBRAIN REGIONS IN ANESTHETIZED RAT DURING MANIPULATION OF LC ACTIVITY.

Effects of LC inactivation on neocortical and hippocampal EEG activity [Publication 11]. Intrabrainstem administration of α_2-agonists into the region of the LC has been observed by others to increase behavioral and EEG measures of sedation. Because these drugs act to inhibit LC neuronal discharge activity and NA release, these observations are consistent with an action of the LC/NA system in the maintenance of an activated forebrain. However, interpretation of results obtained utilizing intratissue drug infusions for the study of LC function is complicated by a variety of factors, such as the small size of the nucleus and the close proximity of the LC to other nuclei known to affect behavioral and EEG states. These factors, together with the absence of electrophysiological measures documenting the relationship between changes in LC neuronal activity and EEG state following such infusions preclude specific conclusions regarding the site(s) of action for the sedative effects of intrabrainstem administered α_2-agonists.

If, in fact, intrabrainstem administered α_2-agonists enhance EEG measures of sedation through an inhibition of LC neuronal discharge activity, it would be hypothesized that: 1) infusions that are effective in suppressing LC activity will alter forebrain EEG; 2) changes in LC neuronal discharge activity will precede changes in forebrain EEG activity; 3) the return of EEG activity to the pre-infusion state will follow the recovery of LC neuronal activity; 4) infusions that are not effective at suppressing LC neuronal discharge activity will not alter forebrain EEG measures.

In these studies, clonidine infusions (35 nl or 150 nl) were made immediately adjacent or approximately 1000 um distant to LC. These infusions were made under conditions in which high-frequency, low-voltage activity predominated in neocortical EEG and theta-activity predominated in hippocampal EEG. The following was observed: 1) cortical and hippocampal activity were not substantially affected following unilateral clonidine-induced LC inactivation; 2) bilateral clonidine infusions that completely suppressed LC neuronal discharge activity in both hemispheres induced a shift in cortical activity to low-frequency, large amplitude activity and the replacement of theta-activity with mixed frequency activity in hippocampus; 3) 35 nl infusions placed 800-1200 um from the LC did not induce a complete suppression of LC activity and did not

alter forebrain EEG; 4) 150 nl infusions placed 800-1200 um from LC were either ineffective at completely suppressing LC neuronal discharge activity or did so with a longer latency to complete LC inhibition and a shorter duration of inhibition; 5) in all cases, the latencies of EEG responses were coincident with the complete bilateral inhibition of LC discharge activity and persisted throughout the period during which bilateral IC neuronal discharge activity was completely absent (60-240 min); 6) the resumption of pre-infusion EEG activity patterns closely followed the recovery of LC neuronal activity or could be induced with systemic administration of the α_2-NA antagonist, idazoxan. These results suggest that the clonidine-induced changes in EEG were dependent on the complete bilateral suppression of LC discharge activity and that, under the present experimental conditions, the LC/NA system exerts a potent and tonic activating influence on forebrain EEG state such that activity within this system is necessary for the maintenance of an activated forebrain EEG state.

Pilot studies: LC manipulations and simultaneous cortical microdialysis. In pilot experiments involving microdialysis, peri-LC bethanechol infusions were made to increase LC neuronal discharge levels in halothane-anesthetized rats that had dialysis probes implanted in frontal neocortex. NA was assayed using HPLC with electrochemical detection. In 12 cases, rats were implanted with dialysis probes 2-3 hours prior to initiation of baseline sample collection. This procedure yielded stable baseline NA levels throughout the experiment which was conducted over the next few hours. In 6 additional cases, rats were implanted with dialysis probes the day prior to the experimental session. This was done because there is evidence that during the first 3-8 hours following dialysis probe insertion, a significant fraction of NA release is impulse independent. In the latter cases, rats were anesthetized with halothane, the dialysis probes implanted, and the rats replaced in their home cages. The following day, the rats were anesthetized with halothane, the LC located, and the experiment conducted exactly as the other 12. In all cases, 2-3 hours following initiation of halothane anesthesia, 3-4 20-min baseline samples were collected. At this point, a bethanechol infusion (1-8 ng/nl) was made at the start of a dialysis sampling interval. At the end of this 20-min sample, a recovery sample was collected, followed by a sample during which the LC was again activated, followed by 1-2 recovery samples. Infusions that increased LC neuronal discharge levels to a maximum of approximately 3 times basal levels, with a total duration of activation of approximately 10 min, resulted in a 50-100% increase in NA in dialysate samples. NA concentrations returned to baseline levels in the sample immediately following LC activation, and comparable NA responses were consistently observed with repeated LC activation. Bethanechol infusions that increased LC neuronal discharge levels to a maximum of approximately 5-6 times basal levels, with a total duration of activation of approximately 15-20 min, also increased NA concentrations 50-100%. Thus, there appears to be a ceiling beyond which increased LC discharge does not result in a corresponding increase in NA release. There are at least 2 possible explanations for this ceiling effect. First, it could result from a rapid depletion of releasable NA during the first few minutes of LC stimulation, with a subsequent recovery over approximately the next 15 min. If this is the case, it would be predicted that the first 10 min of the 20-min sample would contain substantially more NA than the 2nd 10 min, and the 2nd 10 min could contain substantially less NA than preinfusion samples. This has been examined in 3 animals to date. In these experiments, 2 consecutive 10-min samples were collected immediately following LC activation. The results did not indicate that NA release was substantially greater in the first 10 min than in the 2nd 10 min. In these experiments, the second 10-min sample contained NA concentrations that were either quite similar to the first 10-min sample or were intermediate between the first 10-min sample and the recovery sample. These results suggest that releasable NA is not being rapidly depleted following LC activation. A second possible explanation for the ceiling effect is that enhanced NA release results in an activation of presynaptic α_2-NA receptors that inhibit NA release. This can be tested by examining the NA response to moderate- and high- level LC stimulation in the presence of an α_2-antagonist added to the dialysis perfusion buffer. These studies have been initiated (in collaboration with Dr. Ron Kuczenski) and

substantial pilot data have been obtained. Although we have now resolved some initial technical difficulties with the microdialysis methods, the postdoctoral fellow who was conducting these studies, Dr. Craig Berridge, recently left the laboratory. Thus, future of these studies is uncertain.

AIM 2: TO TEST THE HYPOTHESIS THAT LC-INDUCED ACTIVATION OF FOREBRAIN EEG IS MEDIATED BY LC/NA ACTIONS ON CHOLINERGIC AND/OR NON-CHOLINERGIC NEURONS WITHIN BASAL FOREBRAIN NUCLEI.

Involvement of the medial septal region in LC/NA modulation of forebrain EEG. The basal forebrain cholinergic nuclei, located in the substantia innominata/nucleus basalis of Meynert and the medial septal area/diagonal band of Broca are thought to play important roles in the modulation of cortical and hippocampal EEG. These nuclei receive a dense LC/NA innervation. We have initiated a series of experiments designed to determine whether LC/NA influences on forebrain EEG state are mediated by these nuclei. Initially, the effects of the β-agonist, isoproterenol (ISO), and the β-antagonist, timolol (TIM), infused into the medial septum on cortical and hippocampal EEG were examined in halothane-anesthetized rats. In order to perform a preliminary evaluation of this hypothesis, the following 3 questions were examined in approximately 60 rats: 1) What is the effect of ISO when infused into the medial septum under conditions in which slow-wave activity predominates in neocortex and hippocampus? 2) What is the effect of ISO infused outside the region of medial septum? 3) What is the effect of TIM infused bilaterally into the medial septum under conditions in which cortical and hippocampal EEG are activated? 4) What is the effect of TIM infused bilaterally into medial septum on peri-LC bethanechol-induced EEG activation? In these experiments, 26 ga. guide cannulae were implanted over left and right medial septum, penetrating cortex 1.5 mm at an angle of 4° to permit placement of a 33 ga. infusion needle into medial septum while avoiding damage to the superior sagittal sinus and fibers of passage that travel along the most medial aspect of the septal area. Infusions consisted of 100-150 nl of vehicle or drug, at a concentration of 25 ug/ul, infused over a 1-min period. Halothane was adjusted to permit the appropriate level of anesthesia, dummy infusion needles were placed into left and right cannulae and 30-45 min of baseline EEG was collected. 10 min prior to a medial septum infusion, the dummy needle was removed and a needle loaded with 2% Pontamine Sky Blue dye in phosphate buffer saline or drug dissolved in this solution was inserted.

Bilateral vehicle infusions into the medial septum had no obvious EEG effects. In contrast, 1-10 min following 100-150 nl of unilateral ISO, hippocampal theta activity was substantially increased bilaterally and, in the majority of cases, there was a less robust but clear decrease in cortical slow-wave activity. The duration of these responses ranged from 20 min to greater than 60 min and could be reversed with TIM infusion. Identical volumes of ISO infused into the striatum a similar distance from the lateral ventricle had no EEG effects, indicating that these effects of ISO are not due to diffusion into the ventricular system and action at a distant site. Similarly, ISO had no effects on forebrain EEG when infused into the lateral septum or directly into the lateral ventricle approximately 1 mm posterior to the posterior end of medial septum, or into substantia innominata.

Under conditions in which forebrain EEG was in an activated state, unilateral TIM infusion had no EEG effects. In contrast, bilateral TIM resulted in a shift in hippocampal EEG from nearly pure-theta activity to mixed frequency activity and the appearance of large-amplitude, slow-wave activity in cortex.

The effects of TIM on peri-LC bethanechol-induced EEG activation were also examined. In these experiments, peri-LC infusions were made under 3 experimental conditions; prior to any septal infusions, 10 min following bilateral medial septal vehicle infusions, or 10 min following bilateral medial septal TIM infusions. Bilateral TIM blocked or severely attenuated the peri-LC bethanechol-induced activation of cortical and hippocampal EEG.

To summarize, as was observed with unilateral LC activation, unilateral infusions of the β-agonist, ISO, elicited bilateral EEG activation in hippocampus

and cortex. Unilateral medial septal infusions of the β-antagonist, TIM, had no effect on either cortical or hippocampal EEG, whereas bilaterally infused TIM substantially decreased indices of EEG activation in both structures.

One possible explanation for the lack of effects of ISO when infused into substantia innominata on either cortical or hippocampal EEG is that, given the relatively large size of this area and the relatively small infusion volumes, the drug was not diffusing throughout an adequate volume of the structure to elicit EEG changes. Therefore, in an additional 4 cases, unilateral and bilateral ISO infusions were made in which the concentration of the drug was doubled and the infusion volume was either doubled (300 nl) or tripled (450 nl). These infusions had no obvious consistent effects on either hippocampal or cortical EEG.

A full-length manuscript describing these results is now in preparation. We have also prepared sections through the areas in which infusions were made and stained them for the presence of NA or dopaminergic fibers. These anatomical findings will enhance the interpretability of the electrophysiological results.

AIM 3: TO EXAMINE, IN MONKEY, THE EFFECTS OF ACTIVATING OR INACTIVATING THE LC/NA SYSTEM ON EEG AND ON DIALYSIS MEASURES OF THE RELEASE OF NA AND OTHER MONOAMINES IN NEOCORTEX AND HIPPOCAMPUS. THE EFFECTS ON THESE EEG AND DIALYSIS MEASURES OF SYSTEMICALLY ADMINISTERED ADRENERGIC DRUGS THAT ALTER COGNITIVE PERFORMANCE WILL ALSO BE DETERMINED.

The effects of systemically administered adrenergic agents on monkey P300s [Publication 2]. In previous funding periods we have characterized and studied the neural substrates of a monkey event-related potential component that exhibits many of the characteristics of the P3 or P300 components of human event-related potentials. The study to be summarized in this section evaluated the role of NA in the modulation of an auditory version of this P300 response.

In this study, EEG, behavioral, and event-related potential data were collected from squirrel monkeys in an auditory "oddball" paradigm in which the subjects could bar-press for a food reward only during a short interval following the occurrence of target stimuli that were embedded within repetitively occurring non-target stimuli. Data were obtained following the systemic administration of placebo or clonidine, an α_2-noradrenergic agonist that suppresses LC activity and NA release. Clonidine significantly decreased the area and increased the latency of the P300-like potential that occurred following target stimuli, while leaving earlier peaks unaffected. Rates of behavioral responding were not diminished following clonidine administration. This indicates that the suppression of the P300-like potential was not due to sedation.

This report has now been published.

LC neuronal activity in awake monkeys: relationship to spontaneous EEG and auditory P300-like potentials [Publication 3]. These experiments were designed to test the hypothesis that novel auditory stimuli lead to phasic and/or tonic increases in LC discharge activity, which may be a necessary condition for the occurrence of P300 potentials. Event-related potentials and LC unit activity were recorded simultaneously in 3 untrained macaque (Macaca fascicularis) monkeys during the presentation of an auditory oddball paradigm. Oddball stimuli resulted in probability-sensitive, P300-like potentials. While these event-related potential findings are novel, we were able to obtain only a limited number of high-quality LC recordings in this paradigm. Three of 12 LC units showed small phasic enhancements of LC firing after infrequent but not frequent tones. In an additional set of studies, one monkey was trained to bar-press in response to the occurrence of the target stimulus in the oddball paradigm. Interestingly, this animal displayed a prominent P300-like wave, but only when he performed the oddball task accurately. In sessions where the monkey did not respond, neither P300-like potentials nor phasic LC responses were elicited by target stimuli. However, LC cells did tend under these conditions to show a tonic elevation in firing following targets. For the 2 LC neurons whose activity we were able to record during sustained, accurate performance of the task, phasic discharge activity was observed following the presentation of targets, and the timing of this activity indicated it was related to the behavioral response rather than to stimulus presentation. Finally, comparisons of the discharge

activity of individual LC neurons with EEG recordings, under circumstances where no stimuli were presented to these subjects, confirmed our previous observations in squirrel monkey that LC discharge activity is strictly correlated with, and anticipates by 500 to 1000 msec, changes in cortical EEG.

The report describing these results has now been accepted for publication.

Assessment of monoamine release via microdialysis in unanesthetized monkey. We have performed microdialysis in an awake, chair-restrained cynomolgus monkey (Macaca fascicularis) to assess extracellular monoamine concentrations. This involved the development of techniques and equipment, as well as implementing procedures similar to those that would be used in proposed Specific Aim 3. Amphetamine administration was used in these experiments to demonstrate the specificity and sensitivity of the methods.

Because monkeys exhibit large individual differences in brain structure and size, and thus stereotaxic coordinates, for both cortical and subcortical structures, the initial step for this study was to perform an MRI brain scan on this animal to accurately determine the stereotaxic locations of target structures. With the head fixed in a plastic stereotaxic instrument, MRI scans were performed in the 3 standard stereotaxic planes. The resulting images were subjected to a detailed analysis in order to determine the stereotaxic locations of target structures.

After a two-week recovery period, the monkey underwent surgery for the implantation of dialysis guide cannulae and a device for later immobilizing the head. Using aseptic techniques, guide cannulae were cemented in place bilaterally over: 1) primary motor cortex (n=4); 2) parietal cortex (n=4); and 3) the head of the caudate nucleus (n=4). The device that was later used to immobilize the head during chairing also served to hold a protective cap to prevent access by the animal to the cannulae and other parts of the head implant while he was in his home cage. After recovery from surgery, the monkey was habituated to the chairing procedure in daily sessions, including having the head fixed in position for 2-3 hours at a time. Following an additional 1-month period, collection of dialysis samples was initiated. 2-3 dialysis probes were inserted per session, and each session consisted of 4 days of repeated dialysis. On Day 1, the animal was chaired, the head fixed, dialysis probes inserted, the cap replaced, and the animal returned to its home cage. On Day 2, the animal was chaired, the head fixed, probes were connected to the perfusion pump, and sample collection was initiated. After 4 20-minute baseline samples were collected, amphetamine (0.25 mg/kg) was administered subcutaneously, and 3 additional samples were collected. Days 3 and 4 were identical to Day 2. At the end of Day 4, the probes were removed. Sessions were separated from each other by a 1-2 week period. After 5 such sessions, all possible sites had been used, some more than once. At this time, the animal was deeply anesthetized, dye was infused through dialysis probes reinserted through each of the guide cannulae, and the animal was perfused using our standard protocol for immunohistochemical experiments.

In caudate dialysis samples, DA (approx. 100 fmol/sample), DOPAC, HVA, 3MT and 5HIAA were reliably detectable. Amphetamine increased DA concentrations in caudate samples approximately 10-fold. In samples from parietal and motor cortices, DA (approx. 5 fmol/sample), NE (approx. 3 fmol/sample), 5HIAA, DOPAC, HVA and MHPG were all quantifiable. (Our current HPLC assays have limits of quantification, i. e., 3X noise, near 2 fmoles per sample.) Amphetamine increased both NA and DA concentrations approximately 10-fold. In all regions, HVA was present in much higher concentrations than DOPAC, a pattern opposite that observed in rodents. As expected, NA was not detectable in baseline or post-drug samples from caudate.

The results of our attempts to dialyze a single site over several days, or to reinsert dialysis probes into previously used sites, indicated that such an approach is not currently feasible. In both cases, basal dialysate DA, NA, and their metabolites were substantially decreased, in some cases below detection limits, and the responses to amphetamine challenge were significantly diminished, and in some cases no longer evident. We and others have observed similar changes following repeated probe insertions into rodent brain. For these reasons, the proposed studies will use each dialysis site only once.

Histological analyses revealed that the MRI procedure substantially enhanced the accuracy of guide-cannula placement, relative to a purely stereotaxic approach. Most of the probe sites were found to be within 0.5 mm of their intended locations.

To summarize, these results demonstrate the feasibility of applying microdialysis methodologies, and of measuring extracellular DA and NA and their catabolites in cortical and subcortical regions in awake monkey.

We have now completed attempts to obtain dialysis data in an additional 2 monkeys. These results have been mixed, with significant brain infections in both animals. In the next animal, substantially different methods will be used in an attempt to correct this problem.

AIM 4: TO EXAMINE, IN MONKEY, THE EFFECTS OF ACTIVATING OR INACTIVATING THE LC/NA SYSTEM ON NEOCORTICAL AND HIPPOCAMPAL EEG MEASURES AND ON COORDINATED BIMANUAL MOTOR BEHAVIOR.

We have now completed experiments on the first monkey to be used in these studies. Results were mixed. It appears that clonidine infusions were successfully made into the LC region, but our LC recordings were not of sufficient quality and/or reliability to allow us to determine whether these infusions were effective in altering LC discharge activity. However, we are encouraged that we have hardware that appears capable of directing the placement of the LC recording microelectrode and the infusion needle in the appropriate brain region. Also, we have hopefully collected some "control" data demonstrating that infusions into surrounding brainstem regions are ineffective in producing large changes in behavior or EEG. Another monkey has now been implanted and is ready for use in the next series of experiments.

OTHER ACTIVITIES

The results obtained during the past few years in this project have served as the basis for a review article [Publication 5] and for a substantial part of a book chapter [Publication 4].

FULL-LENGTH PUBLICATIONS: YEAR 07

1. Berridge, C.W. and Foote, S.L. Locus coeruleus-induced modulation of forebrain electroencephalographic (EEG) state in halothane-anesthetized rat. Brain Res. Bull., in press.

*2. Swick, D., Pineda, J.A., and Foote, S.L. Effects of systemic clonidine on auditory event-related potentials in squirrel monkeys. Brain Res. Bull. 33:79-86, 1994.

3. Swick, D., Pineda, J.A., Schacher, S., and Foote, S.L. Locus coeruleus neuronal activity in awake monkeys: relationship to auditory P300-like potentials and spontaneous EEG. Exp. Brain Res., in press.

4. Foote, S.L. and Aston-Jones, G. Pharmacology and physiology of central noradrenergic systems. To appear in: Psychopharmacology: The Fourth Generation of Progress. Bloom, F.E. and Kupfer, D.J. (Eds.), Raven Press, New York.

*5. Berridge, C.W., Arnsten, A.F.T., and Foote, S.L. Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological, and behavioral studies in animal models (Editorial). Psychol. Med. 23:557-564, 1993.

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Psychological Medicine, 1993, **23**, 557–564.

EDITORIAL

Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological and behavioural studies in animal models[1]

The early demonstration of the neurotransmitter role of norepinephrine (NE) within the brain stimulated intense study of its functions over the subsequent three decades. A large majority of brain noradrenergic neurons are concentrated in the brainstem nucleus, locus coeruleus (LC). This nucleus gives rise to an extensive and regionally specialized noradrenergic innervation of the CNS, including providing the sole source of NE to hippocampus and neocortex. Despite the intense examination of the LC/noradrenergic system, and the knowledge gained concerning various properties of this system, its functions remain enigmatic.

This editorial will provide a brief review of electrophysiological and behavioural studies which indicate that NE enhances cortical information processing through a variety of actions distributed across multiple anatomical regions and involving multiple receptors. It is proposed that the LC/noradrenergic system acts to create dynamic, widespread patterns of electrophysiological activity in neocortex and other forebrain areas that provide essential substrates for the operation of attentional and other cognitive processes. A greater understanding of the actions of the LC/noradrenergic system may provide the opportunity to treat cognitive dysfunction better in certain patient populations.

CHARACTERISTICS OF THE LC/NORADRENERGIC SYSTEM

The LC is a well-delineated cluster of noradrenergic neurons, located adjacent to the fourth ventricle in the pontine brainstem. It is composed of a small number of neurons (approximately 1600 per nucleus in rat, several thousand in monkey, and 10000–15000 in human). However, these cells possess immensely ramified axons such that the nucleus projects throughout the neuraxis, from spinal cord to neocortex (reviewed in Foote *et al.* 1983).

In rodents, there appears to be little topographic specificity in these projections in the sense that individual LC neurons project to widely dispersed regions. However, within terminal fields noradrenergic axons are characterized by regionally specific patterns of innervation. For example, LC axons preferentially innervate particular cortical regions and laminae, especially in primates (reviewed in Foote & Morrison, 1987). The dense innervation of layer I is particularly interesting, given that this is considered an important site for cortical integration (Vogt, 1991). Heterogeneity is also observed at the receptor level. Thus, traditionally three noradrenergic receptor subtypes have been recognized: α_1, α_2, and β. α_1- and β-receptors are thought to exist primarily at post-synaptic sites whereas α_2-receptors are believed to be present both pre- and post-synaptically; presynaptic α_2-receptors exert an inhibitory influence on LC neuronal discharge and NE release (Svensson *et al.* 1975), whereas the actions of post-synaptic α_2-receptors are largely unknown. The distribution across and within cortical regions varies between the specific receptor subtypes, as does second-messenger coupling. For example, β-receptors appear to be more broadly distributed across cortical laminae and are positively coupled to the Gs/cAMP second-messenger system, whereas α_1- and α_2-receptors are concentrated in the superficial layers and are coupled to the phosphoinositol and

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Gi/cAMP systems, respectively (Dohlman *et al.* 1991). Recently, molecular biological studies have revealed an even greater diversity of adrenergic receptor subtypes indicating additional degrees of complexity (Jones & Palacios, 1991). The distinct cellular functions mediated by the recently identified receptor subtypes remain largely unknown.

To summarize, the LC constitutes a system in which alterations in activity of a very small number of neurons can be broadcast to vast brain regions and neuronal populations of immense number. However, within this system there exists a degree of specificity conferred by the pattern of fibre termination, receptor subtype distribution, and second-messenger coupling that provides a potential substrate for the performance of multiple functions.

These anatomical characteristics suggest possible global actions of the LC/noradrenergic system, such as modulation of behavioural state. This hypothesis is buttressed by recordings from LC neurons in unanaesthetized animals. The pioneering work of Hobson & McCarley and their colleagues demonstrated that these neurons exhibit their highest discharge rates during waking, evince very slow rates during slow-wave sleep, and are silent during REM or paradoxical sleep (Hobson *et al.* 1975). Furthermore, changes in their discharge rates anticipate changes in behavioural state (Hobson *et al.* 1975; Foote *et al.* 1980; Aston-Jones & Bloom, 1981). Subsequent work, by several laboratories, has confirmed and extended these observations. Of particular interest in the present context is the observation that within the waking state, LC neurons discharge most rapidly in anticipation of EEG and behavioural changes that signal enhanced arousal or attentiveness (Foote *et al.* 1980; Aston-Jones & Bloom, 1981). This suggests that enhanced LC activity, which presumably initiates enhanced NE release in forebrain and other target areas, may help to create widespread alterations in forebrain activity patterns that are reflected in EEG measures and are an integral part of epochs of enhanced alertness and particular cognitive functions.

This view is compatible with numerous studies demonstrating a facilitatory effect of NE on information processing by individual cortical and hippocampal neurons. To summarize briefly a vast literature, these studies show that the application of NE to such neurons can reduce spontaneous or weakly driven activity while at the same time preserving or enhancing responses to other, potent, specific synaptic inputs (Foote *et al.* 1975; Woodward *et al.* 1979). Thus activation of LC neurons might induce a state in which forebrain circuits are tuned to provide the greatest possible discrimination between optimal and non-optimal inputs. Clearly, such a scheme suggests an important role for the LC/NE system in attentional and other cognitive processes.

In addition to the ability of NE to enhance signal-to-noise ratios in target regions, a number of electrophysiological actions of the LC/NE system have recently been identified that support a modulatory role of the this system in global, state-dependent cognitive processes. These observations are briefly reviewed below.

FOREBRAIN EEG

The observation that changes in LC neuronal discharge rates precede changes in EEG state implies but does not demonstrate a causal role for the LC in EEG state modulation. To address this hypothesis better, we recently conducted a series of studies in which electrophysiological recordings were used to guide placement of an infusion needle in close proximity to the LC through which drugs that alter LC neuronal discharge rates were infused (Berridge & Foote, 1991). Electrophysiological recordings verified and quantified the effects of these infusions on LC neuronal activity. It was observed that the selective enhancement of LC neuronal discharge resulted in a robust activation of forebrain EEG in anaesthetized rats. This EEG response was blocked by pre-treatment with the β-noradrenergic antagonist, propranolol. Conversely, bilateral inhibition of LC neuronal discharge activity decreased signs of forebrain EEG activation (Berridge *et al.* 1991, 1993). In both the LC activation and inactivation studies, the onset and recovery of the EEG responses closely followed the changes in LC neuronal activity levels. Furthermore, localization studies indicated that infusion-induced changes in EEG state were not observed when infusions were placed

outside the immediate vicinity of the LC. These observations indicate that, under these experimental conditions, the LC is a potent modulator of forebrain EEG state, with LC neuronal activity being causally related to the maintenance of EEG activity patterns associated with arousal.

These results are consistent with *in vitro* observations of McCormick and colleagues that NE enhances neuronal responsivity and induces a shift in firing pattern of cortical and thalamic neurons to a single-spike firing mode that is associated with arousal and/or attention (see McCormick *et al.* 1991). These effects are due to a complicated array of actions of NE at α,- and β-receptors located on numerous cell types. The concerted actions of NE in both cortex and thalamus create a state in which the signal processing ability of forebrain neural systems is enhanced.

ERPs/P300

The responsiveness of LC neurons to stimuli that elicit an orientating response suggests the involvement of these neurons in attention-related cognitive processes. In humans, attention is often studied using event-related potentials (ERPs). ERPs are voltage fluctuations time-locked to sensory, motor, or cognitive events that are extracted from EEG recordings using signal averaging techniques. The P300 component of human ERPs is elicited in response to novel and/or task-related stimuli and is thus thought to have particular relevance to attentional or mnemonic processes.

In monkeys, P300-like components are also observed and have been studied in animal models of attention. The LC/NE system appears to be critical for the generation of a normal P300 response. Thus, bilateral LC lesions in squirrel monkeys selectively decreased P300 components of the ERP (Pineda *et al.* 1989). Similarly, inhibition of LC firing and NE release with systemically administered clonidine also decreases P300 components (Swick *et al.* 1988). These results suggest an important role of the LC/noradrenergic system in the modulation of cortical responsiveness to sensory information.

LTP

Potent modulatory actions of NE have been observed in an extensively studied model of cellular mechanisms of memory, long-term potentiation (LTP). LTP refers to a use-dependent, long-lasting increase in synaptic strength or efficacy: when excitatory synapses are rapidly and repetitively stimulated for brief periods (tetanic stimulation), the post-synaptic neurons generate action potentials more readily upon subsequent stimulation. In the hippocampal formation, three forms of LTP have been described; one involving mossy-fibre input to CA3 of the hippocampus (from the dentate gyrus), one involving the Schaffer collateral input to region CA1 (from the entorhinal cortex), and one involving perforant path input to the dentate gyrus. The observation of LTP in a structure critical for memory function further motivates interest in LTP as a possible mechanism underlying memory.

NE has been demonstrated to influence LTP observed in CA3 and dentate gyrus. Thus, depletion of NE substantially decreases the population spike observed in dentate gyrus (Stanton & Sarvey, 1985), whereas NE application elicits a frequency-dependent enhancement of LTP in the CA3 subfield (Hopkins & Johnston, 1984). These effects appear to be dependent on actions of NE at β-receptors (Hopkins & Johnston, 1988).

NE also elicits a long-lasting enhancement of synaptic efficacy in both the dentate gyrus and CA1 region of the hippocampus *in vitro* in the absence of tetanic stimulation. Thus, NE increases the population spike evoked by perforant path stimulation in the dentate gyrus (Stanton & Sarvey, 1987) and by Schaffer collateral stimulation in the hippocampal CA1 region (Heginbotham & Dunwiddie, 1991). The latter effects were attributable to actions of NE at β-receptors. *In vivo*, similar effects were observed in the dentate gyrus following either LC activation (Harley & Sara, 1992) or NE application (Stanton & Sarvey, 1987). These observations indicate a potentially critical role of the LC/noradrenergic system in mediating long-lasting modifications in synaptic efficacy.

BEHAVIOURAL STUDIES

The above described observations indicate that NE enhances electrophysiological responses of individual neurons and neuronal ensembles, and can induce activity patterns associated with enhanced cortical signal processing throughout the forebrain. Based on these observations, it is posited that one function of the LC/noradrenergic system is the modulation of behavioural state and/or state-dependent processes. The ability of NE to enhance cortical function by reducing 'noise' and/or facilitating the processing of relevant signals suggests that the LC/noradrenergic system might act to enhance cognitive function under 'noisy' conditions where irrelevant stimuli impair performance. Experimental data from rodents, monkeys, and human patients support this prediction.

In rodents, depletion of forebrain NE generally does not impair performance of simple learning and memory tasks: these tests presumably place little demand on the cortex, and NE facilitatory mechanisms may be superfluous. An exception to this rule is the aged (Leslie *et al.* 1985; Collier *et al.* 1988) or in the combined LC/nucleus basalis lesioned animal (Haroutunian *et al.* 1990), suggesting that NE actions may become significant to simple learning in the compromised brain. However, NE depletion does produce deficits in the performance of young, otherwise intact animals on a variety of tasks when irrelevant stimuli are presented during testing. Thus, the addition of distracting visual stimuli at the choice point in a T-maze produces a much greater disruption of performance in NE depleted rats than in sham-treated animals (Roberts *et al.* 1975; Oke & Adams, 1978). Similarly, the presentation of irrelevant, auditory stimuli interrupts the visual discrimination performance of rats with forebrain NE depletion, although these animals perform normally under nondistracting conditions (Carli *et al.* 1983). Further, NE depletion increases conditioned responses to irrelevant stimuli while decreasing responses to relevant stimuli (Lorden *et al.* 1980; Selden *et al.* 1990).

In primates, the prefrontal cortex (PFC) serves a critical role in inhibiting the processing of irrelevant stimuli (Knight *et al.* 1981; Woods & Knight, 1986). In monkeys, this region can be functionally subdivided, with the dorsolateral PFC associated with proper performance in the delayed-response task, a test of spatial working memory. Monkeys with bilateral PFC lesions are markedly and permanently impaired on this task (Goldman-Rakic, 1987) and are especially vulnerable to interference from irrelevant stimuli (Bartus & Levere, 1977).

Experimental evidence collected from non-human primates suggests an important, facilitatory role of NE in the proper regulation of PFC-dependent behaviour through actions at α_2-noradrenergic receptors. Thus, noradrenergic α_2-agonists, such as clonidine or guanfacine, improve performance in dorsolateral PFC-dependent tasks in monkeys with catecholamine (CA) depletion produced by MPTP (Schneider & Kovelowski, 1990), reserpine (Cai *et al.* 1992), local infusion of 6-hydroxydopamine (6-OHDA) into the PFC (Arnsten & Goldman-Rakic, 1985), or ageing (Arnsten & Goldman-Rakic, 1985; Arnsten *et al.* 1988). Further, systemically-administered α_2-agonists lose their cognitive-enhancing effects when the PFC is ablated (Arnsten & Goldman-Rakic, 1985), demonstrating that intact PFC circuitry is necessary for these drugs to have beneficial effects.

Aged monkeys are a useful model for studying NE effects on PFC function because they have naturally occurring catecholamine loss in the PFC (Goldman-Rakic & Brown, 1981), and PFC-dependent cognitive deficits. Thus, aged monkeys are more vulnerable to interference from irrelevant stimuli (Bartus, 1979) and show marked impairment on the delayed response task (Bartus, 1979). Administration of clonidine or guanfacine to aged monkeys markedly improves performance of the delayed response task (Arnsten *et al.* 1988), especially when irrelevant information is present during the delays (Jackson & Buccafusco, 1991; Arnsten & Contant, 1992). These beneficial effects of α_2-agonists are independent of their ability to induce hypotension or sedation (Arnsten *et al.* 1988) and are blocked by α_2-, but not α_1-antagonists (Arnsten & Goldman-Rakic, 1985; Arnsten *et al.* 1988).

Pharmacological studies indicate that enhancement of PFC-related cognitive function results

from stimulation of a subtype of α_2-receptor with high affinity for guanfacine: the Ri site of Boyajian & Leslie (1987), which appears to be the α_2A-site of Bylund (Uhlen & Wikberg, 1991). The gene for this subtype is thought to reside on chromosome 10 (Bylund *et al.* 1992), and pharmacological analysis of the cloned receptor is consistent with a post-synaptic α_2-receptor (Regan *et al.* 1988).

Behavioural pharmacological experiments also indicate that cognitive enhancement results from stimulation of post-synaptic α_2-receptors. Therefore, as mentioned, clonidine has beneficial effects in monkeys with CA depletion, consistent with actions at post- rather than pre-synaptic receptors. Further, low ('pre-synaptic') doses of clonidine actually impair performance in aged monkeys (Arnsten *et al.* 1988), while higher, beneficial doses are blocked by the post-synaptic α_2-antagonists, SKF104078 or SKF104856 (Arnsten & Contant, 1992).

At least some of these beneficial effects of α_2-agonists are due to drug actions directly in the PFC. In aged monkeys, infusion of clonidine into the PFC, but not nearby premotor cortex, improves delayed response performance (unpublished observations). Further, in young monkeys with 6-OHDA lesions restricted to the PFC, clonidine's potency directly relates to the degree of NE depletion in the PFC, consistent with drug actions at supersensitive post-synaptic α_2-receptors in this cortical region (Arnsten & Goldman-Rakic, 1985).

NE stimulation of α_2-receptors appears to be more important for PFC function than for abilities dependent on posterior cortical areas. Thus, α_2-agonists produce marked improvement in aged and young CA-depleted monkeys performing PFC-dependent tasks such as delayed response (Arnsten *et al.* 1988) or delayed match-to-sample (Jackson & Buccafusco, 1991), but have less influence on visual discrimination (Arnsten & Goldman-Rakic, 1985) or delayed nonmatch-to-sample (Arnsten & Goldman-Rakic, 1990) tasks. These latter tasks are thought to depend primarily on inferior and medial temporal lobe function, respectively.

CLINICAL IMPLICATIONS

One implication of the experimental observations described above concerns cognitive dysfunction associated with normal ageing. Humans display marked decrements in performance on PFC-dependent tasks with ageing, similar to those observed in monkeys. For example, Davis *et al.* (1990) observed age-related impairments in performance on the Stroop Interference and Wisconsin Card Sort tests, even at relatively early stages in the ageing process. Thus, the ability of α_2-agonists to improve performance of PFC-dependent tasks in aged monkeys suggests the possibility that these drugs may be of benefit in the treatment of cognitive decline associated with ageing in humans. However, this hypothesis remains to be tested.

That α_2-agonists can exert beneficial effects on PFC-dependent abilities in humans has been documented in studies of patient populations with cognitive disorders not related to ageing. For example, clonidine improved PFC-dependent function in patients with Korsakoff's amnesia (Stroop Interference test, Word Fluency and Memory Recall) (Mair & McEntee, 1986; Moffoot *et al.* 1992), schizophrenia (Trails B, memory recall (Fields *et al.* 1988)), and attention deficit disorder (attention regulation and impulsiveness) (Hunt *et al.* 1985). Extensive studies of Korsakoff's patients have shown that clonidine's ability to improve memory correlates with the degree of NE loss as indicated by CSF MHPG, consistent with an action of clonidine at post-synaptic α_2-receptors (McEntee & Mair, 1990). Most notably, recent SPECT imaging studies of Korsakoff's patients have revealed that clonidine's ability to improve word fluency is significantly correlated with increased regional cerebral blood flow in the PFC (Moffoot *et al.* 1993). These observations indicate that α_2-agonists selectively enhance PFC function in human as well as non-human primates. Finally, analogous to monkeys, cognitive performance of humans with cortical lesions due to Alzheimer's disease is not improved by α_2-agonists (Schlegel *et al.* 1989), demonstrating the need for intact PFC circuitry.

FUTURE DIRECTIONS

To summarize, results from various investigations of LC/NE function reveal a surprising degree of cohesion: whether at the level of the single cell, populations of cells, or whole animal (behaviour), NE increases responses to relevant stimuli (signal) and/or facilitates suppression of responses to irrelevant stimuli (noise). Thus, an emerging view is one in which the LC/noradrenergic system exerts widespread effects at multiple levels of neuronal organization that facilitate cortical processing of sensory information and enhance cognitive processes that guide behaviour. At present, the evidence suggests that global changes in cortical efficiency may be mediated by α_1- and β-receptors, whereas more specific cortical actions (e.g. within PFC) may involve post-synaptic α_2-receptors. Additional studies are needed to address this hypothesis.

An implication of the hypothesis that optimal cognitive and behavioural performance is dependent on actions of the LC/noradrenergic system is that malfunction of this system might contribute to cognitive dysfunction associated with certain psychiatric and/or neurological disorders. A major impediment to the testing of this latter hypothesis is a lack of appropriate methodology for the specific manipulation of this system in humans. Although a pharmacological approach is desirable, there are a number of complicating issues associated with this approach.

First, most noradrenergic drugs exert peripheral as well as CNS effects. Many of the peripheral effects are either intolerable or could affect cognitive function through non-specific actions. Secondly, the recent identification of multiple subtypes of β-, α_1- and α_2-receptors complicates matters, at least in the short term, in that the majority of currently available drugs do not display a high degree of selectivity for these recently identified subtypes.

Future studies will provide critical information concerning the multiplicity of noradrenergic receptor subtypes and structure–function relationships of these subtypes. This information is essential for the design of subtype-selective agonists and antagonists that exert minimal effects in the periphery. In turn, these drugs will provide the tools to improve the study of cognitive functions subserved by individual receptor subtypes. It is hoped that a clearer picture of the role(s) of individual receptors and the pharmacological tools to manipulate these receptors specifically will lead to the development of treatments targeted for specific cognitive dysfunction.

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Effects of Systemic Clonidine on Auditory Event-related Potentials in Squirrel Monkeys

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SWICK, D., J. A. PINEDA AND S. L. FOOTE. *Effects of systemic clonidine on auditory event-related potentials in squirrel monkeys.* BRAIN RES BULL 33(1) 79–86, 1994.—Event-related potential (ERP), electroencephalographic (EEG), and behavioral data were collected from squirrel monkeys (*Saimiri sciureus*) in a 90–10 auditory oddball paradigm. Background or target tones were presented once every 2 s, and responses to the targets were rewarded. ERPs were recorded from epidural electrodes following systemic administration of clonidine (0.1 mg/kg) or a saline placebo. EEG power spectra and behavioral performance were assessed simultaneously as indices of behavioral state. Clonidine significantly decreased the area and increased the latency of a P300-like potential. The amplitudes and areas of the earlier P1, N1, and P2 components and a later slow wave-like potential were not reduced, nor were their latencies altered. Clonidine produced increased EEG power in the alpha range (7.5–12 Hz) and decreased power in the upper beta range (20–40 Hz) but did not affect performance in the oddball task. Because two major effects of clonidine are to substantially reduce activity in the noradrenergic nucleus locus coeruleus (LC) and to reduce norepinephrine (NE) release from axons, the present results support the hypothesis that the LC and its efferent projection system are important in modulating the activity of P300-like potentials.

P300 Event-related potentials Locus coeruleus Norepinephrine Clonidine

THE P300 is a widely studied "endogenous" component of the event-related potential (ERP) recorded from the human scalp. It is a positive potential that peaks approximately 300–600 ms after the presentation of novel or target stimuli embedded in a repetitive sequence of background events (46,47,50). Unlike short latency "exogenous" components, the P300 seems relatively insensitive to physical parameters of the stimulus but instead is responsive to subjective probability, stimulus meaning, and task relevance [see (12,41) for reviews]. Many psychological constructs have been invoked to explain the cognitive processes associated with P300, including context-updating, stimulus categorization, and orienting of attention, although no consensus has been reached [see (11,54) for discussion].

An equally difficult problem has been determining which neural structures generate P300. The concept of a single, anatomically discrete generator is no longer tenable, given the large number of brain regions implicated in its electrogenesis. Clinical studies in humans with brain lesions have been suggestive of specific neural generator sites in frontal cortex and temporal-parietal junction (29,30). Depth recordings in humans have demonstrated late positive potentials similar to P300 (and in some cases, polarity reversals) in hippocampus and medial temporal lobe (MTL) structures (22,31,48), frontal lobe (45,56), parieto-occipital junction (28), inferior parietal lobe (45), and thalamus (58). These findings are somewhat inconclusive, because it is difficult to determine whether a particular intracranial potential contributes to a simultaneous potential recorded on the scalp (55). MTL lesions, for example, do not alter scalp-recorded P300 in a manner consistent with an MTL primary generator (25,26,49).

Another approach has been to develop animal models using cats, rabbits, and monkeys to more systematically examine possible neural substrates (1,6,19,36–38). Recent studies have started to elucidate the role of neurotransmitter systems in modulating P300 activity. Lesions of the septo-hippocampal cholinergic system in cats result in a transient increase followed by a progressive decrease and disappearance of P300-like activity (23). Consistent with this finding, the anticholinergic drug scopolamine decreases the amplitude and increases the latency of P300 in humans (32). Lesions of the noradrenergic nucleus locus coeruleus (LC) in squirrel monkeys also produce a significant reduction of P300-like potentials (39).

The LC, located in the pontine brain stem, is one of several subcortical transmitter systems that modulates neocortical function and behavioral state, along with cell groups containing acetylcholine, dopamine, and serotonin [reviewed in (18)]. One hypothesized function of the LC is a crucial involvement in the control of attention, arousal, and response to novel events (2,17). The LC has a highly divergent efferent projection system that

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innervates widespread regions of primate neocortex with regional and laminar specificities (33,34). LC neurons increase their firing rates following the presentation of novel stimuli which elicit an orienting response, and changes in LC unit activity precede changes in EEG and behavioral state (3,4,16,20,42). These anatomical and physiological characteristics led to the hypothesis that the LC is involved in the production of P300.

To extend the prior observation that LC lesions decrease auditory P300-like potentials in squirrel monkeys (39), we administered the alpha-2-noradrenergic agonist clonidine, which suppresses LC unit activity (8), to squirrel monkeys and recorded ERPs in an auditory oddball paradigm. Systemic administration of clonidine at doses comparable to the one used here substantially inhibits LC firing in awake cats and monkeys (20,42,43). Because sedation is a side effect of clonidine (57), EEG power spectra and behavioral performance were assessed concurrently as indices of behavioral state. A preliminary study in humans (14) indicated that clonidine decreases P300 amplitude in an auditory discrimination task, while early results from our laboratory suggested that a similar phenomenon occurs in monkeys (52).

METHOD

Subjects

Subjects were six adult male Guyanan squirrel monkeys (*Saimiri sciureus*) approximately 4–8 years old and weighing 700–1000 g. Prior to this study, all subjects had received extensive conditioning and training in the auditory oddball paradigm described below.

Surgical Procedures

Monkeys were initially anesthetized with ketamine hydrochloride (40 mg/kg IM) and placed in a Kopf stereotaxic instrument. Anesthesia was maintained with halothane (0.5–1.5%) mixed with oxygen (2.5 l/min) administered via an endotracheal tube. Using aseptic surgical procedures, the skin overlying the dorsal aspect of the skull was resected, the underlying muscles were retracted, and small stainless steel screws (00 gauge, 1.5 mm long) were threaded into burr holes in the skull at locations analogous to those of the 10–20 International System. Wire leads from these electrodes were attached to a multipin connector, and then electrodes, leads, and connector were attached to the skull with dental acrylic. Dilaudid (0.02 mg/kg IM) was administered as necessary to relieve postoperative discomfort. Recording sessions were begun after an uneventful 14-day recovery period.

Recording Protocol

During recording sessions, monkeys were seated in a specially designed primate chair that restricted gross bodily movements and placed inside an electrically shielded, sound-attenuating chamber. A flexible cable was attached to the connector on the monkey's head to record EEG signals from midline (Fz, Cz, Pz) and lateral (F3, F4, P3, P4) electrodes. These were placed over cortical areas analogous to those underlying similar sites in humans (35). For example, the three midline electrodes were placed over frontal cortex (Fz), anterior to the central fissure (Cz), and over posterior parietal cortex (Pz). Electrodes were also implanted in the bony orbit dorsolateral to both eyes to monitor eye movements. All sites were referenced to an electrode implanted approximately 1 mm below the inion. Eight channels of EEG were amplified by a Grass model 7D polygraph with 7P5B preamplifiers having a bandpass of 0.15 to 35 Hz. EEG activity time-locked to stimulus presentation was digitized online from 100 ms before to 900 ms after stimulus onset at a sampling rate of 256 Hz by an IBM-AT compatible computer and stored on disk for subsequent analysis. Free-field auditory stimuli were presented through a small speaker centered approximately 15 cm above the monkey's head.

Oddball Paradigm

During ERP recording sessions, subjects were presented with tone pips (100 ms duration, 70 dB SPL) once every 2 s in a pseudorandom sequence, with the only constraint being that two targets could not occur consecutively. Background tones (3 kHz) occurred 90% of the time, while target tones (1 kHz) occurred 10% of the time for a total of 1000 trials per session. The subjects were trained to press a lever between 200 and 900 ms after the targets to receive fruit juice.

Monkeys were initially conditioned to associate target tones with a reward. Early training sessions consisted of approximately 200 trials and were gradually lengthened. For a period of 4 to 6 months, an experimenter manually rewarded [illegible]nkeys with juice and raisins after target tones were pre[illegible]uring this time, the animals were introduced to the lev[illegible]llowed to shape their own behavior. The total amount of [illegible]ng received prior to this study varied from 6 months to 2 years.

Drug Administration

All monkeys received an injection of clonidine HCl (0.1 mg/kg IM) or a saline placebo several minutes after being seated in the chair. This dose of clonidine was most effective in decreasing P300-like activity in a preliminary dose–response study (52). Two subjects received drug first, while the others received placebo first. Recording sessions began 15 min after drug administration. At least 3 weeks separated the two treatments.

Data Analysis

ERP averages for each condition (placebo and clonidine) and stimulus type (target and background) were computed for individual subjects; grand averages were computed across subjects. Difference waveforms were derived by subtracting ERPs to background stimuli from ERPs to targets. An artifact rejection program automatically excluded trials with voltage levels that exceeded ±75–100 microvolts. Latencies and amplitudes of ERP components were quantified using computer programs that measured the largest positive (P) or negative (N) peaks relative to 100 ms of prestimulus baseline within intervals determined by both preliminary visual inspection of the data and previously reported values (38). Area (ms-μV) under the curve within the specified intervals was also measured for all components. The intervals were: a) P1: 20–120; b) N1: 40–140; c) P2: 80–200; d) P250: 200–400; e) P573: 400–700.

The same EEG epochs utilized for ERP analyses were also processed to determine power spectra. These were decomposed into component frequencies by utilizing a fast Fourier transform algorithm. The resulting spectral density functions were combined into a composite average. Integrated power, mean power, and percentage of total area were measured for each of the following bands: a) delta: 0.5–4 Hz; b) theta: 4–7.5 Hz; c) alpha1: 7.5–9; d) alpha2: 9–12; e) beta1: 12–20; f) beta2: 20–40. The data were statistically evaluated using analyses of variance (ANOVA) with repeated measures.

RESULTS

ERP Measures

Placebo. ERPs recorded after placebo administration resembled baseline ERPs previously reported for squirrel monkeys in

an active auditory oddball paradigm (38). Grand average waveforms across all six subjects are shown in Fig. 1. These ERPs were averaged across all stimuli, whether or not the subjects responded. The initial triphasic P1-N1-P2 complex was most prominent at Fz, showing an anterior-to-posterior gradient along midline sites. Mean peak latencies were: P1, 59 ms; N1, 86 ms; P2, 162 ms.

Although P1 amplitude did not show a significant main effect for probability ($p > 0.1$), there was a significant probability by electrode interaction, $F(6, 30) = 4.47$, $p < 0.003$. Student's t-tests at individual sites showed that P1 responses were significantly larger to infrequent than to frequent tones only at Fz ($p < 0.04$). No main or interactive probability effects were observed for N1 or P2 amplitude nor for N1 or P2 area. Latency and amplitude values are listed in Table 1, area measures in Table 2.

A late positive component (P300-like potential) peaked at approximately 250 ms, showed a relatively broad distribution across all electrode sites, and was significantly larger to targets than to background for area, $F(1, 5) = 7.60$, $p < 0.05$, but not for peak amplitude measures, $F(1, 5) = 4.23$, $p < 0.1$. A very late positive component (Slow Wave-like potential) peaked at 573 ms, had a more posterior and lateral distribution, and was also significantly larger to targets than to background for area measures, $F(1, 5) = 6.71$, $p < 0.05$.

Clonidine. Grand average waveforms for all subjects following clonidine administration are shown in Fig. 1. When drug was included as a factor, P1 amplitude showed a significant main effect for probability, $F(1, 5) = 6.87$, $p < 0.05$, as did P1 area, $F(1, 5) = 8.72$, $p < 0.04$. Clonidine had no other significant effects on latency, amplitude, or area measures for P1, N1, or P2.

There was a 33 ms increase in P250 peak latency with clonidine [Fig. 2; $F(1, 5) = 12.18$, $p < 0.03$]. Clonidine also differentially affected P250 area elicited by target and background tones [Fig. 2; drug × probability, $F(1, 5) = 12.68$, $p < 0.02$]. A pairwise comparison indicated a significant decrease in P250 area for targets ($p < 0.05$) but not for background ($p > 0.4$). Thus, the significant probability effect for P250 area observed with placebo was eliminated by clonidine. There were no main or interactive effects on P250 amplitude.

While there was a tendency for P573 area to be decreased by clonidine, this trend did not reach significance ($p > 0.2$). A separate ANOVA at parietal sites, where P573 was most prominent, also failed to find a significant effect of drug ($p > 0.4$). The probability effect for P573 area was still observed when drug was included as a factor ($p = 0.05$). P573 latency was unchanged ($p > 0.6$). The effects of clonidine on the magnitudes of all components are summarized in Fig. 3 and illustrate the specific effects of clonidine on P250.

Behavioral Performance

Clonidine did not affect performance in the oddball task in a statistically significant way. Although subjects received extensive conditioning and training in the oddball paradigm, they never reached a minimal response criterion (60% correct) for discriminating between the two auditory stimuli. Mean response rates, reported as total percentage of target or background stimuli to which animals responded, were 17.0% (±3.8) correct, 17.0% (±4.7) incorrect for the placebo condition and 13.7% (±4.7) correct, 18.5% (±6.1) incorrect for the clonidine condition. Therefore, a mean total of 170 responses occurred during the placebo session and a mean of 161 during the clonidine session.

EEG Power Spectra

Clonidine generally increased the percentage of total area of the available spectral density function accounted for by the alpha bands and decreased percent area in the beta bands (Fig. 4). Significant increases in both alpha bands were observed at Cz, Pz, F3, and F4 ($p < 0.04$) Significant decreases in beta2 were seen at Pz, P3, P4, and F4 ($p < 0.05$). No significant changes were observed at Fz.

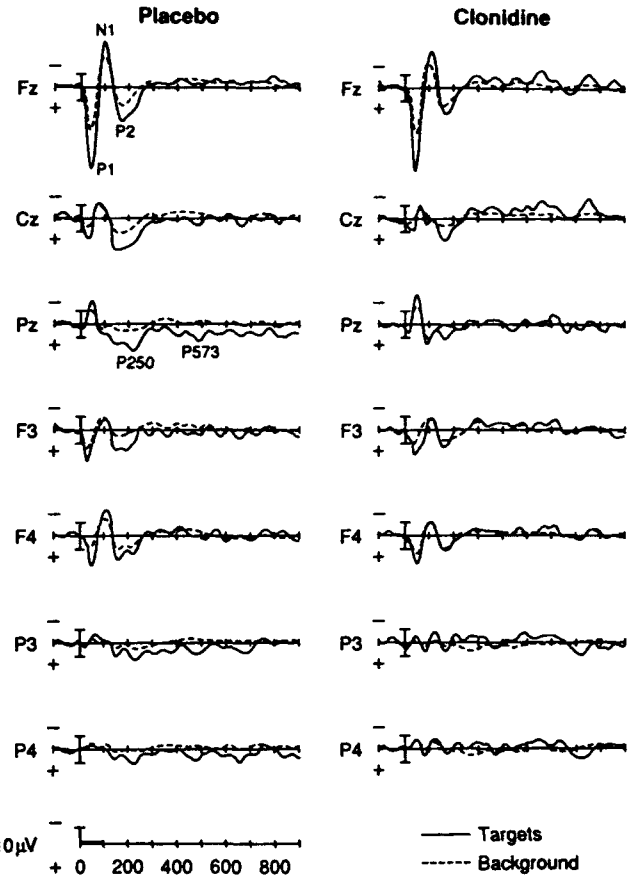

FIG. 1. Left: grand average ERPs across six subjects following placebo administration. ERPs were recorded in response to background (dashed lines) and target (solid lines) tones presented in the 90–10 oddball paradigm. Right: grand average ERPs for the same six subjects following administration of clonidine (0.1 mg/kg IM). ERPs were recorded 15 min postdrug; placebo and drug sessions were separated by at least 3 weeks. Note the dramatic decrease in the magnitude of P250 and P573 while the earlier potentials were unchanged.

DISCUSSION

ERP Measures

ERPs recorded in the auditory oddball paradigm following placebo administration exhibited a long latency, long duration positive potential that peaked at 250 ms. This potential was larger in response to targets than to background tones and showed a relatively broad distribution on the brain surface. Earlier studies have shown that a similar late positive component in squirrel monkeys is sensitive to novelty, stimulus probability, stimulus sequence, task relevance, and behavioral response (35,37,38), suggesting similarities to human P300 potentials. While P250 area in the present study showed a probability effect, P250 amplitude did not. There are three possible explanations for this finding. a) P250 is a unitary component with substantial latency variability. b) There are multiple peaks within the 200–400 ms

TABLE 1

MEAN LATENCIES (ms) AND AMPLITUDES (μV) OF ERP COMPONENTS FOR TARGET (T) AND BACKGROUND (B) STIMULI IN THE 90–10 ODDBALL PARADIGM

		Fz	Cz	Pz	F3	F4	P3	P4
				Latency				
Placebo								
P1	T	49	56	72	49	42	67	70
	B	47	52	78	50	39	70	79
N1	T	109	82	51	92	109	82	87
	B	93	83	56	88	95	83	88
P2	T	170	164	154	161	161	146	153
	B	168	170	170	163	160	162	168
P250	T	236	238	237	276	266	266	232
	B	280	222	239	246	228	269	269
P573	T	556	546	542	541	581	606	555
	B	582	563	582	599	552	617	599
Clonidine								
P1	T	51	65	100	61	64	88	79
	B	49	63	103	57	62	79	83
N1	T	115	70	53	101	97	91	92
	B	107	82	54	92	93	85	63
P2	T	178	170	171	170	166	166	169
	B	168	154	182	163	164	169	168
P250	T	283	261	267	273	275	314	325
	B	277	266	335	242	273	285	285
P573	T	620	569	518	565	550	546	558
	B	536	569	549	575	593	562	582
				Amplitude				
Placebo								
P1	T	68.5	28.1	13.2	29.0	32.2	2.9	4.6
	B	30.9	13.2	12.1	16.3	13.0	0.9	−2.4
N1	T	−37.3	−22.8	−18.1	−18.5	−24.9	−12.6	−8.4
	B	−28.9	−18.3	−13.8	−19.3	−19.3	−8.8	−7.9
P2	T	34.7	33.4	24.2	23.3	26.8	12.4	13.0
	B	17.8	17.0	5.5	9.9	13.2	4.6	2.6
P250	T	22.3	23.7	25.3	20.2	16.8	15.2	12.3
	B	−1.7	4.1	4.6	5.3	5.4	4.5	2.5
Clonidine								
P1	T	72.0	25.8	16.7	33.8	42.2	9.3	1.6
	B	42.1	19.0	12.1	22.9	30.0	7.3	−0.4
N1	T	−31.5	−19.2	−29.6	−22.1	−25.4	−13.0	−13.9
	B	−19.8	−16.0	−18.1	−17.0	−20.7	−6.1	−6.3
P2	T	22.4	18.8	11.5	16.7	19.1	5.1	2.4
	B	15.3	6.6	6.5	11.3	16.0	5.5	1.2
P250	T	4.5	11.7	4.5	6.7	7.6	6.6	7.5
	B	4.5	0.5	4.6	3.9	7.0	7.7	5.7

latency window. Either of these two possibilities increases the difficulty of selecting one peak to be designated as the P250. Additionally, the monkeys did not perform the task with a high degree of accuracy, contributing to the variability across subjects. For this reason (and because the small number of trials yielded an inadequate average), no attempt was made to compare ERPs from correct and incorrect responses. c) A third possibility is that the area measure filtered out high frequency noise present in the peak amplitude measure (which approached significance), resulting in greater statistical power.

A very late, sustained positive potential (P573) resembled the Slow Wave previously observed in humans (44,47). P573 was larger to targets than to background and had a more posterior and lateral distribution than P250. Like Slow Wave, P573 was negative at Fz, minimal at Cz, and positive at parietal sites. As reported in previous studies (38,39), P1 amplitude showed a probability effect which, in the present experiment, was significant only at Fz. This effect could be the result of using the lower pitched tone as the target or is perhaps a reflection of the refractory nature of this component. A significant increase in P1 amplitude is observed when ISI is lengthened (15,37), consistent with a long recovery cycle.

Administration of the alpha-2 noradrenergic agonist clonidine, which inhibits the firing rates of LC neurons, specifically

decreased the area of P250 elicited by target tones. Clonidine also significantly increased the latency of P250 by 33 ms, while the latencies of all other components were unchanged. The amplitudes and areas of N1 and P2 were not affected, nor was P573 area. These findings agree with a preliminary dose–response experiment, which indicated that clonidine produces a dose-related decrease in P250 area, with recovery to control levels in postdrug sessions (52). Clonidine either decreased (0.05 mg/kg) or abolished (0.075 and 0.1 mg/kg) P300-like waves in two trained squirrel monkeys and one untrained monkey. In the present study, clonidine actually enhanced the P1 probability effect, because a significant main effect was observed when drug was included as a factor. Because a cholinergic brain stem-thalamic system appears to modulate auditory P1 (7,10), clonidine may have indirect actions on this system as well.

These results in the auditory modality differ from recent findings in the visual modality. The same dose of clonidine did not affect the latency, amplitude, or area of a monkey P300-like component recorded in a passive visual oddball paradigm (40). In fact, there was a nonsignificant trend for clonidine to increase positive amplitudes in the 150–500 ms time window, particularly at midline electrode sites. Variations in the regional and laminar distribution of NA fibers (18) and receptors (5) may contribute to differential NA modulation of P300-like activity elicited by auditory and visual stimuli. Lesion data from human patients suggest that P300 has modality-specific generators (26). Because the LC-NA system appears to make a modality-dependent contribution to the generation of P300-like potentials, it could contribute to such effects through distinctive influences on the processing of signals from different sensory modalities.

The present results agree with the findings of Duncan and Kaye (14), who demonstrated that clonidine decreases P300 amplitude in humans performing an auditory discrimination task. The greatest decrease in P300 was observed in response to target tones with the largest frequency separation from background tones (64 Hz), in comparison to tones with intermediate (32 Hz) and small (16 Hz) separations. Clonidine also decreased P300 amplitude in a report by Joseph and Sitaram (27), although only over occipital and left parieto-temporal regions. The current re-

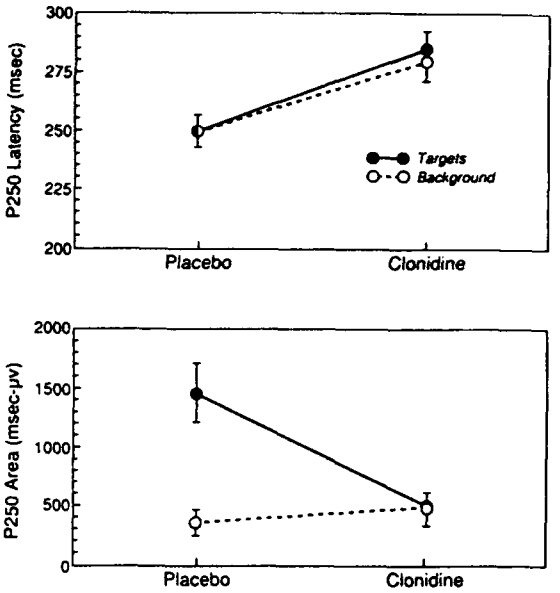

FIG. 2. Top: P250 latency (across all electrode sites) following either placebo or clonidine administration. Clonidine increased P250 latency in response to both targets and background. Bottom: P250 area (across all electrode sites) after placebo or clonidine. Note that clonidine decreased P250 area in response to targets but not to background.

TABLE 2

MEAN AREAS (ms-μV) OF ERP COMPONENTS RECORDED FOLLOWING TARGET AND BACKGROUND TONES IN THE 90–10 ODDBALL PARADIGM

Area		Fz	Cz	Pz	F3	F4	P3	P4
Placebo								
P1	T	2488	987	577	1100	1400	239	309
	B	1026	375	158	521	357	68	32
N1	T	−1452	−896	−631	−624	−1137	−570	−378
	B	−1083	−737	−549	−627	−764	−450	−523
P2	T	1963	2057	2032	1398	1585	745	900
	B	927	955	434	426	632	229	165
P250	T	1184	1410	2080	1641	1033	1544	1250
	B	314	362	412	338	361	466	358
P573	T	191	822	3160	1356	1230	1217	1688
	B	15	148	227	346	483	209	216
Clonidine								
P1	T	2718	605	581	1012	1255	371	281
	B	1511	324	427	531	692	337	66
N1	T	−1126	−494	−921	−457	−574	−511	−602
	B	−723	−538	−649	−394	−556	−235	−440
P2	T	1031	1092	1155	731	803	479	377
	B	749	427	668	398	593	571	197
P250	T	650	522	370	550	562	490	563
	B	471	200	528	319	545	848	739
P573	T	997	816	757	1013	835	1172	911
	B	283	208	307	314	748	647	750

sults, combined with the finding that electrolytic lesions of the LC decrease squirrel monkey P300-like potentials (39), support the hypothesis that the LC is involved in the generation or modulation of auditory P300-like activity.

Behavioral Performance

Several limitations were present in utilizing squirrel monkeys as experimental subjects. These monkeys have a high metabolic rate and are very susceptible to dehydration, hypoglycemia, and stress-related diseases. Brief water deprivation schedules (2 h) were used at the beginning stages of training but did not substantially improve response rates in most cases. Similarly, mildly negative reinforcement for incorrect responses served more to disrupt than to shape behavior and, therefore, was not used. The monkeys' variable performance and failure to discriminate between the tones diminish the importance of the behavioral data, except to indicate that the subjects were not asleep during the clonidine condition.

Nevertheless, the subjects had received extensive conditioning to associate the targets with a reward. In some respects, the ERPs more closely resembled those elicited in an active task (38) than those recorded in a passive paradigm (37). Rather than having a restricted distribution over lateral parietal sites, as is typical for squirrel monkeys in the passive paradigm, P250 exhibited a broad distribution. Whether P250 is more accurately designated a passive P3a or an active P3b remains open for interpretation, however. Clonidine did not alter the frequency of responding, suggesting that the ERP alterations observed in this study were not the result of profound sedation.

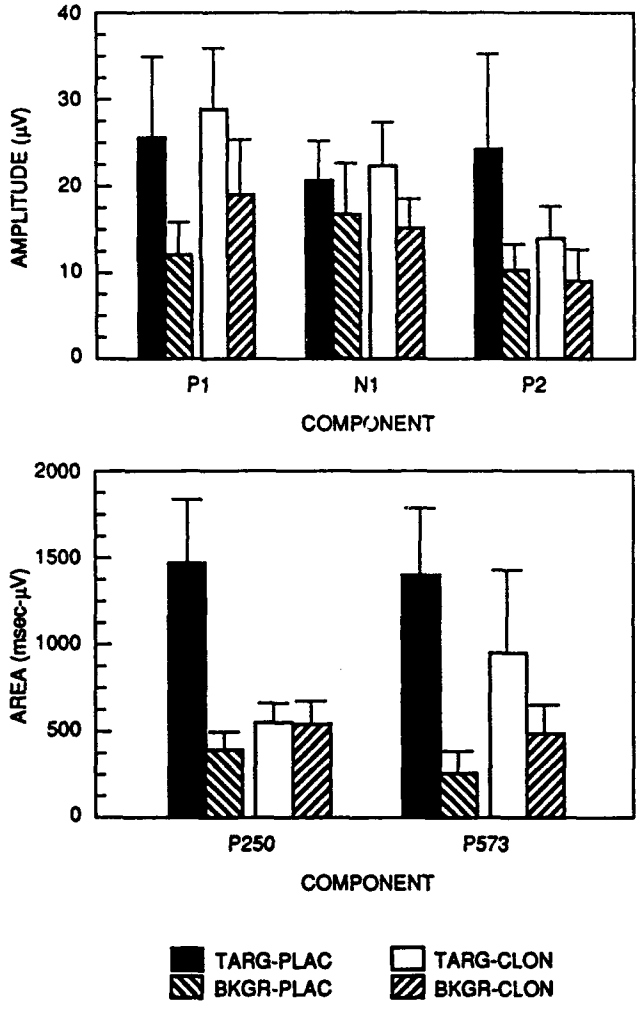

FIG. 3. Effects of clonidine on the amplitudes of P1, N1, and P2 and the areas of P250 and P573. Targets and background are shown for both placebo (TARG-PLAC and BKGR-PLAC) and clonidine (TARG-CLON and BKGR-CLON).

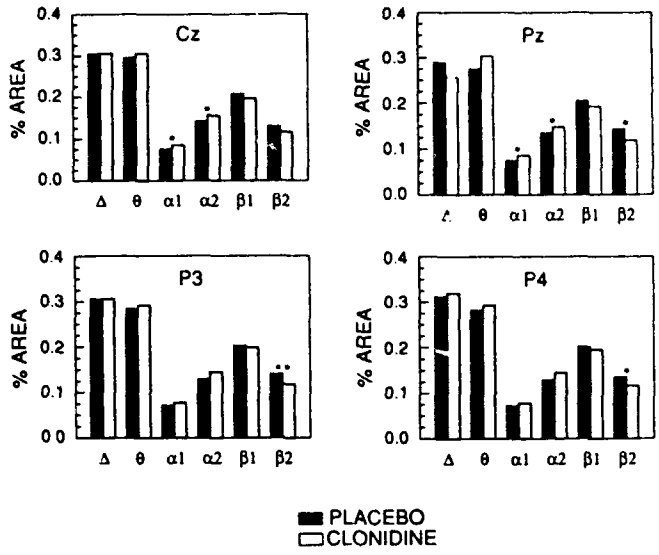

FIG. 4. Percentage of total area within each EEG frequency band for placebo and clonidine conditions. Power spectra were obtained from the same EEG epochs utilized for ERP analyses. The bands are: delta, 0.5–4 Hz; theta, 4–7.5; alpha1, 7.5–9; alpha2, 9–12; beta1, 12–20; beta2, 20–40. Statistically significant changes are noted: $^{*}p < 0.05$, $^{**}p < 0.01$.

EEG Power Spectra

Clonidine produced increased power in the alpha bands and decreased power in the beta2 band during the oddball paradigm, suggestive of a less aroused behavioral state. Spontaneous EEG was not recorded, however, and the presence of ERPs in the power spectra complicates interpretation of these data. The lack of changes in response rate and in performance accuracy argue against the proposal that clonidine's effects on P250 were solely due to sedation.

Sites of Action

Sedation, however, is one of the most common side-effects of clonidine and appears to be mediated by alpha-2 receptors located on LC cell bodies. Microinfusion of clonidine directly into the LC produces slow wave sleep and increased power in the lower frequency bands (9). This does not occur if clonidine is infused into hippocampus, amygdala, thalamus, or cortex. Clonidine-induced sedation can be reversed with the alpha-2 antagonists yohimbine, piperoxan, and phentolamine, while the specific alpha-1 antagonist prazosin has no effect (13).

Peripherally administered clonidine could act at a number of alpha-2 receptor sites, both pre- and postsynaptic. Autoradiographic (59) and radioligand binding studies (53) have shown that alpha-2 receptors are found throughout the brain. Moreover, systemic clonidine indirectly inhibits serotonin neurons

in the dorsal raphe (51), regularizes the normal bursting firing pattern of ventral tegmental dopamine neurons (21), and inhibits histamine release (24). Despite these nonselective effects, two major actions of clonidine are to suppress LC neuronal activity and to diminish NE release via actions on presynaptic NE terminals. Although other mechanisms of action cannot be ruled out, the current results are consistent with the proposal that decreased LC activity interferes with the generation of P300-like potentials.

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